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System Biology of Archaeal RNAs and RNA-Binding Proteins

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It has been proposed that the last universal common ancestor (LUCA) was a thermophile or a hyperthermophile. The hyperthermophilic archaeon *Pyrococcus* species thrives at temperatures above 95°C and is thought to be one of the evolutionarily ancient organisms. Thus, analyzing genome-wide gene functions in *Pyrococcus* could provide new insights into the fundamental knowledge of life. It has been also hypothesized that RNA played a central role in the very beginning of life (the RNA world hypothesis). Therefore, studying gene regulation at the RNA level of the organism may contribute to a better understanding of the basis of gene function.

Our strategy has been focusing on the discovery of the novel RNA-binding proteins and RNA-related enzymes in *P. furiosus*. In a previous study, we systematically screened a recombinant protein library for *P. furiosus* proteins that can bind to RNA. We would like to introduce some of our unique examples of RNA-binding proteins and RNA-related enzymes. Here, we found a novel RNA-binding protein FAU-1 that had a weak amino acid similarity (25%) with that of *Escherichia coli* RNase E (1). We found that a flavin-dependent thymidylate synthase (Pf-Thy1) possessed RNA-binding activity (2). We identified one gene PF0027, which encodes a protein with heat-stable cyclic nucleotide phosphodiesterase (CPDase) activity. We found that the purified PF0027 protein possessed GTP-dependent RNA ligase activity (3).

We have been also studying the transfer RNA (tRNA) evolution in archaea. The origin and evolution of tRNA is one of the important subjects that is discussed in the field of molecular evolution. Recently, three types of tRNAs have been discovered in the archaeal genomes: "non-intronic tRNA", which is encoded on a single gene with no intron sequence; "intron-containing tRNA", which is encoded on a single gene with maximum three introns (4) and "split tRNA" which is produced from two or three separate genes (5). In this talk, I will summarize our findings related to the phylogeny, structure and processing

machinery of all known types of archaeal disrupted tRNAs. Possible evolutionary scenarios of tRNA gene (6) and possible translocations of the archaeal tRNA introns during their evolution will be discussed.

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Disrupted tRNA gene diversity and possible evolutionary scenarios

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Recently, various types of unusual tRNAs have been discovered in the genomes of Archaea and primitive Eukaryotes; multiple-intron-containing tRNAs that possess more than one introns; split tRNAs that are produced from two pieces of RNAs transcribed from separate genes; tri-split tRNAs that are produced from three separate genes; and permuted tRNA in which its 5' and 3' halves are encoded within a permuted orientation in a single gene. All these disrupted tRNAs can form mature contiguous tRNA and be aminoacylated after processing via cis- or trans-splicing. The discovery of such tRNA disruptions has raised a question of when—and why—these complex tRNA processing pathways have emerged through the evolution of life. Many previous reports have noted that tRNA genes contain a single intron at the anticodon loop region, a